Hemodynamic Effects of Local Anesthesia and its Possible Correlation with Chromogranin A

Maha T. Al–Saffar[1], Tahani A. AL-Sandook[2], Mahmoud Y. Taha[1,*]

[1]Department of Dental Basic Science, College of Dentistry, Mosul University, Mosul, Iraq
[2]Iraq Cultural Attachi, Washington, USA
*Corresponding author: tahadent04@yahoo.com

Abstract **Aim:** To study the effects of local anesthesia on blood pressure in patients undergo extraction of maxillary teeth under infiltration local anesthesia and to compare the level of salivary chromogranin A before and after administration of local anesthesia and its possible correlation with hemodynamic effects of local anesthesia. **Materials and Methods:** A total of 26 patients (18 female, 8 male) who need tooth extraction under infiltration local anesthesia were examined in two situations (pre and post local anesthetic administration). For each patient salivary sample was collected by salivette to estimate the level of chromogranin A using ELISA Kits. For all patients, the blood pressure and pulse rate were measured before and after administration of local anesthesia using automatic blood pressure recorder. **Results**: the results showed significant differences between salivary chromogranin A for patients before the administration of local anesthesia (580.33 ± 130.42) ng/ml and after the administration of local anesthesia (674.51+ 130.93) ng/ml with p < 0.05. The results also showed that the mean value of blood pressure (systolic and diastolic blood pressure) lowered after administration of local anesthesia but with no significant differences (p > 0.05) with pre –local anesthetic value. The pulse rate was increase in it is value after local anesthetic administration with a significant differences (p < 0.05) than pre – anesthetic value. **Conclusions**: tooth extraction and administration of infiltration local anesthesia caused hypotension with no significant effect (p > 0.05) while it caused increase in pulse rate with a significant effect (p < 0.05). Administration of infiltration local anesthesia caused significant effects on salivary chromogranin A levels, also this study showed that there was significant inverse correlation between systolic blood pressure and chromogranin A levels(p < 0.05) after administration of infiltrations local anesthesia during dental treatments (tooth extraction).

Keywords: *salivary chromogranin A, local anesthesia, blood pressure*

1. Introduction

LA is a temporary loss of sensation including pain in one part of the body produced by a topically applied or injected agent without depressing the level of consciousness. [1] Dental anxiety and fear of pain associated with dentistry are relatively stable over time. Despite advances in dental equipment, procedure and preventive measures, dental anxiety is a confounding problem with which dentist has to cope [2].

Salivary chromogranin A was shown to be biomarker of acute stress. It was the first granin to be isolated and characterized as uniquely acidic protein co stored and co released with the catecholamine hormones from the bovine adrenal medulla. [3] It was recently demonstrated that chromogranin A and B each regulates the concentration of adrenalin in chromaffin granules and its exocytosis. [4] In contrast to catecholamines which are removed relatively quickly from the circulation by cellular uptake or catabolism. Circulating CgA is relatively stable,

so it has therefore been proposed as a useful marker of sympathetic nervous system activity. [5,6] CgA is expressed and released from serous acinar and ductal cells of the human submandibular salivary gland [7].

The relationship between mean arterial blood pressure (MAP), Cardiac output (CO) and total peripheral resistance (TPR) can be described in Figure 1 and equation.

$$MAP = CO \ TRP$$

Aim: to study the effects of local anesthesia on blood pressure in patients undergo extraction of maxillary teeth under infiltration local anesthesia and to compare the level of salivary chromogranin A before and after administration of local anesthesia and a possible correlation with hemodynamic effects of local anesthesia.

2. Patients, Materials and Methods

This study was carried out at the specialized center for dentistry in Mosul city / Iraq, from February 2013 to July

2013. A total number of patients included in this study were (26) patients with an average age between (20-40) year old.
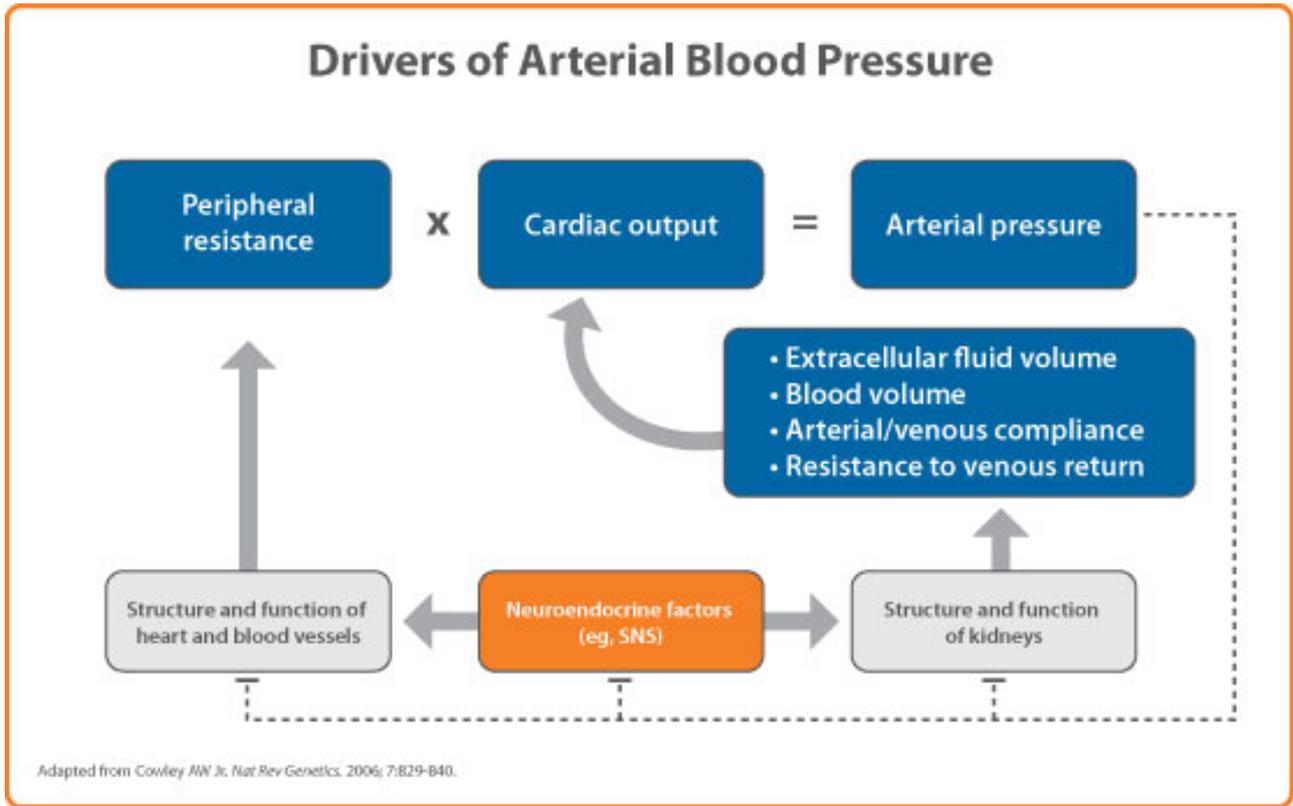

Figure 1. The relationship between mean arterial blood pressure (MAP), Cardiac output (CO) and total peripheral resistance (TPR)

2.1. Criteria of Patients Selection

All patients require infiltration LA injection for dental treatment (extraction) for maxillary teeth only have no history of compromised medical status , no recent use of antibiotics or analgesic drugs (in the last one week), non-pregnant or lactating females, non- smoker, non – alcoholic, and agreed to participate in this study and signed the consent form.

2.2. Study Design and Sample

2.2.1. Patient Grouping

Patients included in this study were examined in two main situations: Pre- anesthetic situation included patients who require a dental treatment (tooth extraction) and were seated on a dental chair for dental and clinical examination **but before taking LA injection.** This group consisted of a total number of (26) patients (8 M, 18 F) with mean of age (20-40) years. Post- anesthetic situation: included patients who were **taking LA Injection** for dental treatments (tooth extraction). This group consisted of total number of (26) patients (8 M, 18 F) with mean age of (20-40) year.

2.3. Drug Used

2.3.1. Local Anesthesia

Local anesthetic cartridges 1.8 ml (lidocaine –hamein 2% with 1:80,000 adrenalin) MOH/ IRAQ were used to produce anesthesia by infiltration to maxillary teeth. [8] The anesthesia was administrated by the researcher only for those patients.

Materials and equipments used for collection, separation and storage of saliva

Equipment	supplier
Salivette	Sarstedt(Germany)
Centrifuge 80-1 TAPLETOP low speed	GSJ, China
Micro pipette 10-100 ml and tips	Jianan, China
Eppendroff tube 1.5 ml	China
Rank for Eppendroff tube	China
Refrigerator	Biofamily, Korea
Freezer	Universal, Korea
Cool box	China

2.4. Salivary Sample Collection and Storage

Unstimulated saliva was collected from patients before and after administration of local anesthesia. Patients were asked to rinse the mouth with 10 ml of tap water to remove food debris then a simple cotton dental roll of specialized devices (Salivette) is sucked or chewed in patients mouth (cheek, floor of mouth, and over the tongue) for a 1 minute allowing the saliva to be absorbed and collected in the swab in an easy and hygienic fashion. All samples were collected between 9 -11 am and should be clear of blood contamination. [9] Salivette then centrifuged at 3000 rpm for 10 min and a clear fluid at the bottom of tube was placed in sterile eppendroff tube and stored at deep freeze _20 Ċ until the time of analysis [10].

2.5. Measuring Blood Pressure and Heart Rate

The blood pressure and heart rate of patients were measured pre- and post administration of local anesthesia

using an automatic blood pressure recorder (Rossmax, China). Blood pressure and heart rate recording were repeated immediately to obtain the mean reading [11].

2.6. Human Chromogranin A Measurement

Human chromogranin A was measured by ELISA kits (My BioSource, USA). The test is based on the quantitative sandwich enzyme immune- assay technique.

2.7. Data Analysis

The data obtained from this study was subjected to the statistical analysis including descriptive and analytic methods. For descriptive way the mean of variance was used, while one way analysis of variance and Duncan's Test for (intra and inter group comparison) matching with 5% level of significance. Paired t-test was used to compare between the study groups, while spearman test was used to estimate the possible correlation between the studying parameters.

3. Results

A total of 26 patients participated in this study. Most of them were females (69.2% females, 30.8% males). The mean age of samples was 22.5 ± 6.68 years for female and 30.5 ± 3.5 years for male with age range 20-40 years (Table 1 and Table 2).

Table 1. Distribution of the study samples

Sex	Number	Percentage	P-value	DF	χ^2
Male	**8**	**(30.8)**			
Female	**18**	*(69.2)*			
Total	26	100	0.7	**2**	**0.556**

Analysis was performed by ANOVA test

Table 2. Descriptive statistic of study samples for both sexes

Sex	Mean of age $\pm$ SD	Minim	Maximum
Females	22.5 ± 6.68	20	40
males	30.5 ± 3.5	20	40

SD: standard deviation

The results of this study showed that the mean amount of chromogranin A in patients before taking local anesthesia was (580.33 ± 130.42) ng/ml while its amount was $(674.51 + 130.93)$ ng/ml in patients after taking local anesthesia (Table 3) with significant differences between them ($p < 0.05$).

Table 3. Mean values of chromogranin A (ng/ml) pre and post administration of local anesthesia

Parameters	Pre-local anesthesia Mean $\pm$ SD	Post-local anesthesia Mean + SD	Difference Mean $\pm$ SD	95% CI of difference	T-value	DF	P-value
ChromograninA nglml	580.33 ± 130.42	$674.51 + 130.93$	-96.17 ± 63.99	$-81.02 - 29.32$	-4.396	25	<0.0001

Analysis was performed using paired –t test

The hemodynamic effects of local anesthesia are shown in Table 4. The mean values of systolic and diastolic blood pressure were reduced after administration of local anesthesia but with no significant differences ($p > 0.05$), while the pulse rate was increased after administration of local anesthesia with a significant differences ($p < 0.05$).

Table 4. The pre and post hemodynamic effects of local anesthesia

Parameters	Pre-local anesthesia Mean $\pm$ SD	Post-local anesthesia Mean $\pm$ SD	Difference Mean $\pm$ SD	95% CI of difference	T-value	DF	P-value
Systolic blood pressure	121.65 ± 20.62	120.96 ± 22.19	-1.69 ± 17.96	$-4.56 - 9.94$	0.764	25	0.4
Diastolic blood pressure	78.92 ± 13.08	77.76 ± 16.75	1.15 ± 16.82	$-5.64 - 7.94$	.350	25	0.7
Pulse rate Pulse/minute	87.88 ± 13.25	89.65 ± 13.19	-2.76 ± 8.09	$-8.03 - 1.50$	-3.006	25	0.006

Analysis was performed using paired t test.

The correlations between the studying parameters performed by pearson correlation and spearman test are shown in Table 5 which demonstrated inverse significant correlation between chromogranin A and systolic blood pressure with significant difference $p < 0.05$.

Table 5. The correlation between chromogranin A, systolic, diastolic blood pressure and pulse rate

	Systolic	Diastolic	Pulse rate
Chromogranin A	**-0.26**[*]	**-0.04**	**-0.05**
	0.02	0.7	0.6

Note: upper bold fig (correlation coefficient r), lower figure (p-value), analysis were performed by pearson correlation test and spearman test

4. Discussion

The lignocaine-adrenaline combination is the most widely used local anaesthetic solution in dental practice. This combination is essential as adrenaline counteracts the known localized vasodilator effects of lignocaine in subcutaneous and submucosal vessels by causing vasoconstriction, thereby acting as a "chemical tourniquet" and thus decreasing the rate of systemic absorption from the site of injection and reducing the risk of anesthetic toxicity. Adrenaline increases the depth and duration of action. In addition, it provides haemostasis for surgical procedures. [12] Our study showed that a low dosage of adrenaline may produce significant hemodynamic effects particularly obvious hypotension combined with slight increase in pulse rate. Many hemodynamic studies were carried out on patients subjected to local anesthesia with vasoconstrictor. [13,14,15,16] No significant changes were recorded in either blood pressure (systolic and diastolic) or heart rate. However, some authors suggested that such changes were dependent upon the injected vasoconstrictor dose. The hypotensive effect of epinephrine at subtherapeutic concentrations was evaluated in a series of reports by Yang et al., (2005). [17] Although both α- and β-adrenocepters are stimulated, β2 vasodilator effect is most sensitive. Thus, in large doses, direct stimulatory effects on cardiac output plus potent vasoconstriction (particularly in precapillary resistance vessels of skin,

mucosa, and kidney) produce a rapid increase in systolic arterial pressure. Diastolic pressure is affected less because of β2 – receptor induced vasodilation in muscle beds (the characteristics physiologic distribution of the circulation associated with adrenaline), therefore the pulse pressure widens.

This results were also in agreement with many other studies like Bortoluzzi et al., (2010) [18] who explained results with regard to adrenaline and adrenergic system stimulation. It might be that adrenaline has both β1 and β2 activity. β1 stimulation tends to cause an increase in blood pressure, whereas β2 stimulation tends to decrease blood pressure, therefore it often does not dynamically increase blood pressure due in part to β2 activity. A second possible explanation is that the hemodynamic alterations are usually short in plasma due to short adrenaline half-life which is approximately less than three minutes. In addition, when stimulated, the sympathetic nervous system primarily releases norepinephrine and secondarily releases epinephrine. The effects of both substances on blood pressure are limited [19,20].

The study of Haghighat et al., (2006) [21] showed a similar results and explained the increase of systolic blood pressure was probably due to stress (pre- administration of local anesthesia) while the lowering of systolic blood pressure after administration of local anesthesia(post-administration of local anesthesia) was probably due to disappearance of patients fear and stress after injection. Most of patients were more worried about injection than the treatment plan. During the injection process, the sight of dental syringe and apprehension of needle stick pain is considered a stressful condition to which in turn stimulates both α1, β2 receptors in blood vessels at the site of injection. They initially cause vasoconstriction as α1 stimulation is predominant. However, subsequent biotransformation, reuptake and redistribution resulting low tissue level making β2 action predominant with resulting vasodilation [22].

Another proposed mechanism for the occurrence is based on activation of the Bezold-Jarisch reflex (BJR). This reaction may be related to venous pooling caused by the sitting position and epinephrine-induced β2-adrenergic effect and increased inotropy (adrenergic effect of epinephrine). Increased epinephrine levels may occur endogenously from decreased venous return and carotid baroreceptor stimulation as well as exogenously from epinephrine administered with the local anesthetic or in the irrigating solution. In this setting, a low-volume hypercontractile ventricle causes stimulation of intramyocardial mechanoreceptors (C fibers) followed by an abrupt withdrawal of sympathetic outflow and an increase in vagal tone with resultant bradycardia and hypotension. [23] Similar results were obtained by Shaban et al, (2013) [24] and explained the increase in pulse rate after administration of local anesthesia due to the vasodilating effects of lidocaine .

The measurement of salivary chromogranin A (CgA) concentration has been developed as a method to evaluate psychological stress .The use of saliva rather than blood has obvious advantages including the ease of collection and noninvasiveness (painless) which is particularly important for pain and stress assessment. [25] Mitsuhata et al., (2013) [26] hypothysized that pre-treatment CgA values indicated the stress associated with dental treatment and post-treatment CgA values indicated relief from stress after the treatment with no significant correlation between them.
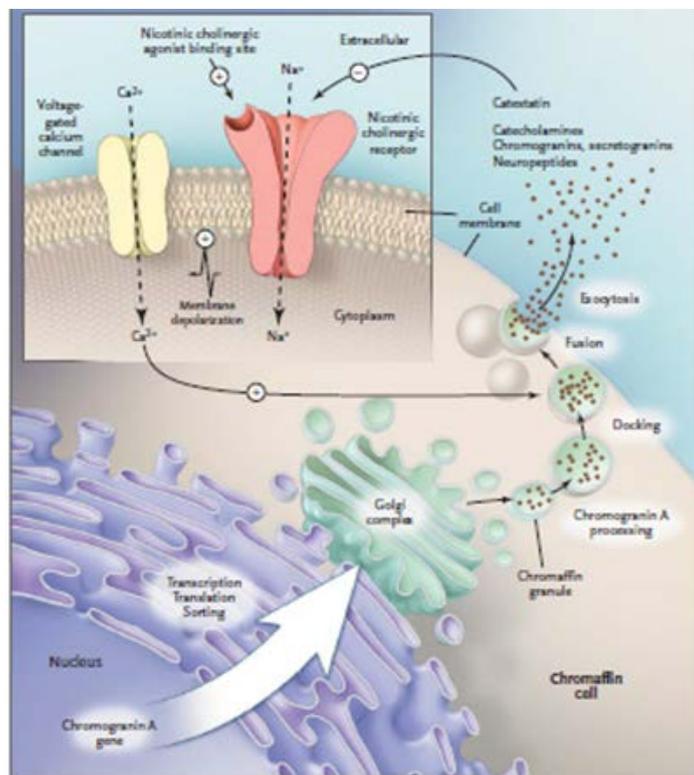

Figure 2. Catestatin role in regulation of catecholamine secretion

There was no previous study on the effects of local anesthetic in dentistry on salivary CgA. According to the results of this study higher amount of CgA after administration of local anesthesia was observed with a significant difference from patients before administration of local anesthesia. The possible explanation of this higher

amount is that the injection pressure of local anesthesia causes pain due to several factors influence pressure including volume injected into tissue per unit time, permeability of the injected solution into bone, soft tissue, blood vessels, and effects of tissue pressure and stretching of the movable mucosa caused by volume of injected solution. [27] The pain and fear of pain are positively related to stress both during pain and in the anticipation of pain so that the fear of pain would be related to higher stress and pain intensity and to reduce placebo analgesia. [28] CgA is considered a biomarker of acute stress which causes activation of sympatho- adreno – medullary system and releases of CgA which has half life of 15-20 minutes. [29] The results of this study showed inverse correlation between CgA and systolic blood pressure and this could be explained by the fact that catestatin (a peptide fragment of the catecholamine secretory vesicle protein chromogranin A) is a potent inhibitor of exocytotic catecholamine secretion from PC12 and chromaffin cells. This peptide acts as a noncompetitive nicotinic cholinergic antagonist with characteristic inhibitory effects on nicotinic cationic (Na1, Ca21) signal transduction. Indeed, catestatin is more potent than substance P in inhibiting nicotine-induced secretion of catecholamines. [30] Catestatin is the first known endogenous compound able to inhibit in vitro catecholamine release from both chromaffin cells and noradrenergic neurons by acting as a non-competitive nicotinic cholinergic antagonist. [31] Angelone et al., (2008) [32] hypothesized that circulating levels of catestatin decreased in the plasma of patients with essential hypertension. Genetic ablation of the chromogranin A (*Chga*) gene in mice increases blood pressure and pretreatment of *Chga*-null mice with Cts prevents blood pressure elevation indicating a direct role of Cts in preventing hypertension. This is in agreement with the study of Tsigelny etal (2013) [33]. Catestatin inhibits the release of catecholamines from sympathoadrenal chromaffin cells by blocking the neuronal nicotinic cholinergic receptor which is the physiologic trigger for secretion [34] (Figure 2).

Catestatin also prevents the desensitization of catecholamine release from chromaffin cells that is induced by repeated nicotinic-agonist stimulation. Thus, catestatin may contribute to an autocrine negative-feedback mechanism that modulates catecholamine release within the sympathoadrenal system. Since excess sympathetic activity was implicated in the development of hypertension, a disturbance of the catestatin mechanism may be a contributing factor. O'Connor et al., (2002) [35] showed that the plasma catestatin level is diminished in patients with hypertension and even in normotensive persons at genetic risk for hypertension. The observation that a low catestatin level is correlated with increased adrenal epinephrine secretion and augmented pressor responses to sympathoadrenal stressors provides further evidence of a link between diminished catestatin and hypertension.

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